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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Arntzen *et al.*

Application No.: 09/129,298

Filed: August 5, 1998

Group Art Unit: 1649

Examiner: O. Zaghmout

For: THE USE OF MIXED DUPLEX OLIGONUCLEOTIDES TO EFFECT LOCALIZED GENETIC CHANGES IN PLANTS

Attorney Docket No.: 7991-023

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Keith Allen Walker, Ph.D., reside at 13315 Roxton Circle, San Diego, CA, 92130, and I hereby declare as follows:

1. I am a scientific investigator and manager of technical research and development. My work has focused on the physiology and molecular biology of plants for over twenty years. I have been involved in plant tissue culture, plant genetic engineering, and commercial applications for plant biotechnology. I now hold the title of President, Plant and Industrial Products Division, KIMERAGEN, Inc., the assignee of the captioned application (the "Application").

2. Prior to my employment by KIMERAGEN, Inc., I was employed by the MYCOGEN CORPORATION as the Director of Business Development and Licensing, Executive Director of Biotechnology Research, and Executive Director of Planning and Licensing. From 1989 to 1992 I served as the Executive Director of Development and Licensing for the AGRIGENETICS Company. In 1981 I was a Co-Founder of PLANT GENETICS, Inc., and served initially as the Director of Product Development, and later as the Vice President and Director of Research. I began my professional career with

MONSANTO AGRICULTURAL PRODUCTS Co., as a Senior Research Biologist, Research Group Leader, and, finally as a Senior Research Group Leader. A copy of my *curriculum vitae* is attached as Exhibit A.

3. My professional research interests have focused upon a number of areas including whole plant physiology, plant tissue culture, plant cell biology, somatic cell genetics and the development and implementation of screening programs.

4. As a consequence of my own experience, and reading the scientific literature concerning the overall field of plant biotechnology, I am very familiar with the technical capabilities typical of those who routinely perform research in the fields of plant genetics, plant molecular biology and plant genetic engineering (hereinafter "one skilled in the art").

5. I am familiar with the contents of the Application, which discloses the use of mixed duplex oligonucleotides for the introduction of localized genetic changes into plants. I am also aware of the fact that an Office Action, mailed August 2, 1999, at the sentence bridging pages 3 and 4, has rejected all claims of this patent application, based upon the allegation that the specification of that application "while enabling for a method for making a localized mutation in the gene which encodes the selectable marker ALS1 and ALS2, and in the gene which encodes the scorable marker GFP using the recombinagenic oligonucleobase, does not reasonably provide enablement for making the localized mutation in non-selectable or non-scorable genes." I interpret a "non-selectable" or "non-scorable," in this context, to be a mutation that cannot be selected or identified at a single-cell level. In contrast the acetolactate synthase (ALS) and green fluorescent protein (GFP) mutations that are exemplified in the Application are selectable and scorable, respectively. These mutations, which are more properly called selectable or scorable markers, are discussed below.

6. I am familiar with the examples disclosed in the Application relating to the introduction of selectable mutations into the gene encoding ALS, thereby providing resistance to chlorosulfuron, as well as to those examples demonstrating reversion of an inactivating mutation in a gene encoding GFP. It is my opinion that these examples, combined with the teaching of this application as a whole, are more than sufficient to enable

one of ordinary skill to create localized mutations causing a desired trait in a plant cell, even though that trait does not provide a selectable or scorable marker.

7. It is my opinion that one skilled in this art could find a cell carrying the desired non-selectable or non-scorable marker by screening procedures of the type that are routinely used in the field in light of the observed frequency with which localized mutations are created in the disclosed processes. For example, the present application reveals the detection of eight cells carrying corrected defects in a mutant GFP gene within a screened population of 300,000 protoplasts, a frequency of conversion of 8 in 300,000 or 1 in 37,500. The frequency with which the desired, non-selectable mutation was observed is within the screening capability of those skilled in this art. Although, in the GFP example the detection method involved observation under ultraviolet light, more labor-intensive methods are, nevertheless, routinely used by investigators in this field.

8. My own experience as the Executive Director of Biotechnology Research at the Mycogen Corporation provides evidence that the screening of tens of thousands of plant samples is considered mere routine experimentation by those in the plant development field. In the early to mid 1990's, we undertook to develop variants of Canola (*Brassica napus*), Corn (*Zea mqys*), Peanut (*Anachis hypogea*), and Sunflower (*Helianthus annus*) having superior properties as oil seed. In these programs chemical mutagens, *e.g.*, ethylmethanesulfonate, were used to produce mutations in seed. The mutagenized seed was planted and progeny seed harvested and non-destructively assayed to identify mutants having the desired properties. The technical details and the results of these programs are described in United States patent No. 5,684,232, No. 5,948,954 and No. 5,965,755, copies of which are attached (Exhibits B-D). To carry out this work we established a laboratory facility that routinely analyzed the oil content about 60,000 samples per month using two operators and five gas chromatographs. Two operators and five instruments is not a scale of resources that is greater than is commonly available to develop novel, commercially valuable plant varieties. Thus, it is my personal experience that routinely available resources would be more than adequate to detect variant plants at the frequencies that can be expected based on the data in the Application.

9. A further example of the screening of tens of thousands of plants as a part of a single project can be found in a recent publication (Tsugane *et al.,* The Plant Cell 11, 1195-1206 (Exhibit E)). A total of 148,300 plants were screened to find two isolates carrying the desired marker. In this experimental approach, 22,300 lines were mutagenized with either ethyl methane sulfonate or treatment with T-DNA tagging. Seedlings were germinated on a low-salt mineral medium and, after 2 weeks, were transferred to a high-salt medium. Therefore, it is evident that those skilled in this area can, and do, screen tens or even hundreds of thousands of isolates to find those cells or plants having a desired phenotype when selection at a single-cell level is not practical.

10. Accordingly, based upon my knowledge, experience and familiarity with the present invention and with the scope of efforts routinely used by those skilled in the art, I would respectfully disagree with the assertion that the scope of enablement of Application is limited to scorable or selectable markers. It is my opinion, based upon the information summarized in paragraphs above, that one skilled in the art would be able to practice the invention described in the Application and by use of resources routinely available to those working in the plant development introduce a mutation that causes any desired trait, even a trait that could be detected only by growing and screening a whole plant or plant seed.

11. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Date 12/28/99 _____ _____

Keith Allen Walker, Ph. D.

Attachments:
Exhibit A: *curriculum vitae of* Keith Allen Walker, Ph. D.
Exhibit B-D: United States patent No. 5,684,232, No. 5,948,954 and No. 5,965,755.
Exhibit E: Tsugane *et al.* (1999) The Plant Cell 11, 1195-1206.